

Sub C

WHAT IS CLAIMED IS:

W B

5 1. A method for detecting pregnancy in a bovine animal comprising:
 (a) obtaining a sample from said animal; and
 (b) detecting at least one pregnancy associated antigen (PAG) wherein said PAG
 is present in early pregnancy and absent at about two months post-partum;

10 whereby the presence of the PAG indicates that said animal is pregnant.

20 2. The method of claim 1, wherein said PAG is selected from the group consisting of
 PAG2, PAG4, PAG5, PAG6, PAG7 and PAG9.

15 3. The method of claim 1, wherein said sample is saliva, serum, blood, milk or urine.

4. The method of claim 3, wherein said sample is saliva.

5. The method of claim 3, wherein said sample is serum.

25 6. The method of claim 3, wherein said sample is blood.

7. The method of claim 3, wherein said sample is milk.

8. The method of claim 3, wherein said sample is urine.

30 9. The method of claim 1, wherein said detecting comprises immunologic detection.

10. The method of claim 9, wherein said immunologic detection comprises detection
 of BoPAG2, BoPAG4, BoPAG5, BoPAG6, BoPAG7, BoPAG9, boPAG 7v;

W B C

6664501 have260

5 11. The method of claim 9, wherein said immunologic detection comprises detection of BoPAG2, BoPAG4, BoPAG5, BoPAG6, BoPAG7, BoPAG9, boPAG 7v, boPAG9v; boPAG 15; boPAG 16; boPAG 17; boPAG 18; boPAG 19; boPAG 20 or boPAG 21 with polyclonal antisera.

10 12. The method of claim 9, wherein said immunologic detection comprises ELISA.

13. The method of claim 9, wherein said immunologic detection comprises RIA.

14. The method of claim 9, wherein said immunologic detection comprises Western blot.

15 15. The method of claim 3, wherein the PAG is BoPAG2.

16. The method of claim 3, wherein the PAG is BoPAG4.

20 17. The method of claim 3, wherein the PAG is BoPAG5.

18. The method of claim 3, wherein the PAG is BoPAG6.

19. The method of claim 3, wherein the PAG is BoPAG7.

25 20. The method of claim 3, wherein the PAG is BoPAG9.

21. The method of claim 3, wherein the PAG is BoPAG7v.

30 22. The method of claim 3, wherein the PAG is BoPAG9v.

23. The method of claim 3, wherein the PAG is BoPAG15.

24. The method of claim 3, wherein the PAG is BoPAG16.

5 25. The method of claim 3, wherein the PAG is BoPAG17.

26. The method of claim 3, wherein the PAG is BoPAG18.

27. The method of claim 3, wherein the PAG is BoPAG19.

10 28. The method of claim 3, wherein the PAG is BoPAG20.

29. The method of claim 3, wherein the PAG is BoPAG21.

15 30. The method of claim 1, further comprising detecting a second PAG in said sample.

31. The method of claim 30, further comprising detecting a third PAG in said sample.

20 32. The method of claim 12, wherein said ELISA is a sandwich ELISA comprising binding of a PAG to a first antibody preparation fixed to a substrate and a second antibody preparation labeled with an enzyme.

25 33. The method of claim 32, wherein said enzyme is alkaline phosphatase or horseradish peroxidase.

34. The method of claim 32, wherein said first antibody preparation is monoclonal.

35. An antibody composition that reacts immunologically with BoPAG2, BoPAG4, BoPAG5, BoPAG6, BoPAG7, BoPAG9, boPAG 7v; boPAG9v; boPAG 15; boPAG 16; boPAG 17; boPAG 18; boPAG 19; boPAG 20 or boPAG 21.

5 36. An antibody composition that reacts immunologically with BoPAG2.

37. An antibody composition that reacts immunologically with BoPAG4.

38. An antibody composition that reacts immunologically with BoPAG5.

10 39. An antibody composition that reacts immunologically with BoPAG6.

40. An antibody composition that reacts immunologically with BoPAG7.

15 41. An antibody composition that reacts immunologically with BoPAG9.

42. An antibody composition that reacts immunologically with BoPAG7v.

43. An antibody composition that reacts immunologically with BoPAG9v.

20 44. An antibody composition that reacts immunologically with BoPAG15.

45. An antibody composition that reacts immunologically with BoPAG16.

25 46. An antibody composition that reacts immunologically with BoPAG17.

47. An antibody composition that reacts immunologically with BoPAG18.

30 48. An antibody composition that reacts immunologically with BoPAG19.

49. An antibody composition that reacts immunologically with BoPAG20.

50. An antibody composition that reacts immunologically with BoPAG21.

5 51. A hybridoma cell that secretes a monoclonal antibody that reacts immunologically with BoPAG2, BoPAG4, BoPAG5, BoPAG6, BoPAG7, BoPAG9, boPAG 7v; boPAG9v; boPAG 15; boPAG 16; boPAG 17; boPAG 18; boPAG 19; boPAG 20 or boPAG 21.

10 52. A method of making a monoclonal antibody to BoPAG2, BoPAG4, BoPAG5, BoPAG6, BoPAG7, BoPAG9, boPAG 7v; boPAG9v; boPAG 15; boPAG 16; boPAG 17; boPAG 18; boPAG 19; boPAG 20 or boPAG 21 comprising:

15 (a) immunizing an animal with a BoPAG preparation;

(b) obtaining antibody secreting cells from said immunized animal;

(c) immortalizing said antibody secreting cells; and

(d) identifying an immortalized cell that secretes antibodies that bind immunologically with the immunizing BoPAG.

20 53. A method of identifying a pregnancy associated glycoprotein (PAG) that is an early indicator of pregnancy in an Eutherian animal comprising:

25 (a) obtaining a cDNA library prepared from the placenta of said animal between days 15 and 30 of pregnancy; and

(b) hybridizing said library under high stringency conditions with a PAG-derived nucleic acid probe;

whereby hybridization of said probe identifies said PAG.

666750-1975260

54. A method of identifying a pregnancy associated glycoprotein (PAG) that is an early indicator of pregnancy in an Eutherian animal comprising:

5 (a) obtaining an RNA preparation from the placenta of said animal between days 15 and 30 of pregnancy; and

10 (b) performing RT- PCR™ on said preparation using PAG-derived primers; whereby amplification identifies said PAG.

10 55. An isolated and purified BoPAG2 polypeptide.

15 56. The polypeptide of claim 55, wherein said polypeptide comprises the sequence of SEQ ID NO:25.

15 57. An isolated and purified BoPAG4 polypeptide.

20 58. The polypeptide of claim 57, wherein said polypeptide comprises the sequence of SEQ ID NO:27.

20 59. An isolated and purified BoPAG5 polypeptide.

25 60. The polypeptide of claim 59, wherein said polypeptide comprises the sequence of SEQ ID NO:28.

25 61. An isolated and purified BoPAG6 polypeptide.

30 62. The polypeptide of claim 61, wherein said polypeptide comprises the sequence of SEQ ID NO:29.

30 63. An isolated and purified BoPAG7 polypeptide.

- 64. The polypeptide of claim 63, wherein said polypeptide comprises the sequence of SEQ ID NO:30.
- 65. An isolated and purified BoPAG9 polypeptide.
- 66. The polypeptide of claim 65, wherein said polypeptide comprises the sequence of SEQ ID NO:32.
- 67. An isolated and purified BoPAG7v polypeptide.
- 68. The polypeptide of claim 67, wherein said polypeptide comprises the sequence of SEQ ID NO:40.
- 69. An isolated and purified BoPAG9v polypeptide.
- 70. The polypeptide of claim 69, wherein said polypeptide comprises the sequence of SEQ ID NO:42.
- 71. An isolated and purified BoPAG15 polypeptide.
- 72. The polypeptide of claim 71, wherein said polypeptide comprises the sequence of SEQ ID NO:44.
- 73. An isolated and purified BoPAG16 polypeptide.
- 74. The polypeptide of claim 73, wherein said polypeptide comprises the sequence of SEQ ID NO:46.
- 75. An isolated and purified BoPAG17 polypeptide.

76. The polypeptide of claim 75, wherein said polypeptide comprises the sequence of SEQ ID NO:48.

77. An isolated and purified BoPAG18 polypeptide.

5 78. The polypeptide of claim 77, wherein said polypeptide comprises the sequence of SEQ ID NO:50.

79. An isolated and purified BoPAG19 polypeptide.

10 80. The polypeptide of claim 79, wherein said polypeptide comprises the sequence of SEQ ID NO:52.

81. An isolated and purified BoPAG20 polypeptide.

15 82. The polypeptide of claim 81, wherein said polypeptide comprises the sequence of SEQ ID NO:54.

83. An isolated and purified BoPAG21 polypeptide.

20 84. The polypeptide of claim 83, wherein said polypeptide comprises the sequence of SEQ ID NO:56.

85. An isolated and purified nucleic acid encoding BoPAG2.

25 86. The nucleic acid of claim 85, wherein said nucleic acid comprises the sequence of SEQ ID NO:2

87. The nucleic acid of claim 85, wherein said nucleic acid encodes a BoPAG2 polypeptide comprising the sequence of SEQ ID NO:25.

30

5694504-1157260

88. An isolated and purified nucleic acid encoding BoPAG4.

89. The nucleic acid of claim 88, wherein said nucleic acid comprises the sequence of SEQ ID NO:4

5

90. The nucleic acid of claim 88, wherein said nucleic acid encodes a BoPAG4 polypeptide comprising the sequence of SEQ ID NO:27.

10

91. An isolated and purified nucleic acid encoding BoPAG5.

92. The nucleic acid of claim 91, wherein said nucleic acid comprises the sequence of SEQ ID NO:5.

15

93. The nucleic acid of claim 91, wherein said nucleic acid encodes a BoPAG5 polypeptide comprising the sequence of SEQ ID NO:28.

20

94. An isolated and purified nucleic acid encoding BoPAG6.

95. The nucleic acid of claim 94, wherein said nucleic acid comprises the sequence of SEQ ID NO:6.

25

96. The nucleic acid of claim 94, wherein said nucleic acid encodes a BoPAG6 polypeptide comprising the sequence of SEQ ID NO:29.

97. An isolated and purified nucleic acid encoding BoPAG7.

25

98. The nucleic acid of claim 97, wherein said nucleic acid comprises the sequence of SEQ ID NO:7.

66450-10260

99. The nucleic acid of claim 97, wherein said nucleic acid encodes a BoPAG7 polypeptide comprising the sequence of SEQ ID NO:30.

100. An isolated and purified nucleic acid encoding BoPAG9.

5 101. The nucleic acid of claim 100, wherein said nucleic acid comprises the sequence of SEQ ID NO:9.

102. The nucleic acid of claim 100, wherein said nucleic acid encodes a BoPAG9 polypeptide comprising the sequence of SEQ ID NO:32.

10 103. An isolated and purified nucleic acid encoding BoPAG7v.

104. The nucleic acid of claim 103, wherein said nucleic acid comprises the sequence of SEQ ID NO:39.

15 105. The nucleic acid of claim 103, wherein said nucleic acid encodes a BoPAG7v polypeptide comprising the sequence of SEQ ID NO:40.

20 106. An isolated and purified nucleic acid encoding BoPAG9v.

107. The nucleic acid of claim 106, wherein said nucleic acid comprises the sequence of SEQ ID NO:41.

25 108. The nucleic acid of claim 106, wherein said nucleic acid encodes a BoPAG9v polypeptide comprising the sequence of SEQ ID NO:42.

109. An isolated and purified nucleic acid encoding BoPAG15.

567604947260

110. The nucleic acid of claim 109, wherein said nucleic acid comprises the sequence of SEQ ID NO:43.

5 111. The nucleic acid of claim 109, wherein said nucleic acid encodes a BoPAG15 polypeptide comprising the sequence of SEQ ID NO:44.

112. An isolated and purified nucleic acid encoding BoPAG16.

10 113. The nucleic acid of claim 112, wherein said nucleic acid comprises the sequence of SEQ ID NO:45.

114. The nucleic acid of claim 112, wherein said nucleic acid encodes a BoPAG16 polypeptide comprising the sequence of SEQ ID NO:46.

15 115. An isolated and purified nucleic acid encoding BoPAG17.

116. The nucleic acid of claim 115, wherein said nucleic acid comprises the sequence of SEQ ID NO:47.

20 117. The nucleic acid of claim 115, wherein said nucleic acid encodes a BoPAG17 polypeptide comprising the sequence of SEQ ID NO:48.

118. An isolated and purified nucleic acid encoding BoPAG18.

25 119. The nucleic acid of claim 118, wherein said nucleic acid comprises the sequence of SEQ ID NO:49.

120. The nucleic acid of claim 118, wherein said nucleic acid encodes a BoPAG18 polypeptide comprising the sequence of SEQ ID NO:50.

5

10

15

20

25

30

121. An isolated and purified nucleic acid encoding BoPAG19.

122. The nucleic acid of claim 121, wherein said nucleic acid comprises the sequence of SEQ ID NO:51.

123. The nucleic acid of claim 121, wherein said nucleic acid encodes a BoPAG19 polypeptide comprising the sequence of SEQ ID NO:52.

124. An isolated and purified nucleic acid encoding BoPAG20.

125. The nucleic acid of claim 124, wherein said nucleic acid comprises the sequence of SEQ ID NO:53.

126. The nucleic acid of claim 124, wherein said nucleic acid encodes a BoPAG20 polypeptide comprising the sequence of SEQ ID NO:54.

127. An isolated and purified nucleic acid encoding BoPAG21.

128. The nucleic acid of claim 127, wherein said nucleic acid comprises the sequence of SEQ ID NO:55.

129. The nucleic acid of claim 127, wherein said nucleic acid encodes a BoPAG21 polypeptide comprising the sequence of SEQ ID NO:56.

130. An oligonucleotide comprising at least about 15 consecutive bases of the sequence of SEQ ID NO:9, or the complement thereof.

131. The oligonucleotide of claim 130, wherein said oligonucleotide is about 20 bases in length.

132. An oligonucleotide comprising at least about 15 consecutive bases of the sequence of SEQ ID NO:7, or the complement thereof.

133. The oligonucleotide of claim 132, wherein said oligonucleotide is about 20 bases in length.

134. An oligonucleotide comprising at least about 15 consecutive bases of the sequence of SEQ ID NO:6, or the complement thereof.

135. The oligonucleotide of claim 134, wherein said oligonucleotide is about 20 bases in length.

136. An oligonucleotide comprising at least about 15 consecutive bases of the sequence of SEQ ID NO:5, or the complement thereof.

137. The oligonucleotide of claim 136, wherein said oligonucleotide is about 20 bases in length.

138. An oligonucleotide comprising at least about 15 consecutive bases of the sequence of SEQ ID NO:4, or the complement thereof.

139. The oligonucleotide of claim 138, wherein said oligonucleotide is about 20 bases in length.

140. An oligonucleotide comprising at least about 15 consecutive bases of the sequence of SEQ ID NO:2 or the complement thereof.

141. The oligonucleotide of claim 140, wherein said oligonucleotide is about 20 bases in length.

5

10

15

20

25

30

142. An oligonucleotide comprising at least about 15 consecutive bases of the sequence of SEQ ID NO:39 or the complement thereof.

143. The oligonucleotide of claim 142, wherein said oligonucleotide is about 20 bases in length.

144. An oligonucleotide comprising at least about 15 consecutive bases of the sequence of SEQ ID NO:41 or the complement thereof.

145. The oligonucleotide of claim 144, wherein said oligonucleotide is about 20 bases in length.

146. An oligonucleotide comprising at least about 15 consecutive bases of the sequence of SEQ ID NO:43 or the complement thereof.

147. The oligonucleotide of claim 146, wherein said oligonucleotide is about 20 bases in length.

148. An oligonucleotide comprising at least about 15 consecutive bases of the sequence of SEQ ID NO:45 or the complement thereof.

149. The oligonucleotide of claim 148, wherein said oligonucleotide is about 20 bases in length.

150. An oligonucleotide comprising at least about 15 consecutive bases of the sequence of SEQ ID NO:47 or the complement thereof.

151. The oligonucleotide of claim 150, wherein said oligonucleotide is about 20 bases in length.

505K60 19872260

152. An oligonucleotide comprising at least about 15 consecutive bases of the sequence of SEQ ID NO:49 or the complement thereof.

153. The oligonucleotide of claim 152, wherein said oligonucleotide is about 20 bases in length.

154. An oligonucleotide comprising at least about 15 consecutive bases of the sequence of SEQ ID NO:51 or the complement thereof.

155. The oligonucleotide of claim 154, wherein said oligonucleotide is about 20 bases in length.

156. An oligonucleotide comprising at least about 15 consecutive bases of the sequence of SEQ ID NO:53 or the complement thereof.

157. The oligonucleotide of claim 156, wherein said oligonucleotide is about 20 bases in length.

158. An oligonucleotide comprising at least about 15 consecutive bases of the sequence of SEQ ID NO:55 or the complement thereof.

159. The oligonucleotide of claim 158, wherein said oligonucleotide is about 20 bases in length.

160. A kit comprising:

25 (a) a first monoclonal antibody preparation that binds immunologically to BoPAG2, BoPAG4, BoPAG5, BoPAG6, BoPAG7, BoPAG9, boPAG 7v; boPAG9v; boPAG 15; boPAG 16; boPAG 17; boPAG 18; boPAG 19; boPAG 20 or boPAG 21; and

30

(b) a suitable container means therefor.

161. The kit of claim 160, further comprising:

5 (c) a second monoclonal antibody preparation that binds immunologically to the same BoPAG as said first monoclonal antibody, but wherein said first and said second monoclonal antibodies bind to different epitopes; and

(d) a suitable container means therefor.

10 162. The kit of claim 161, wherein said first antibody preparation is attached to a support.

163. The kit of claim 162, wherein said support is a polystyrene plate, test tube or dipstick.

15 164. The kit of claim 161, wherein said second antibody preparation comprises a detectable label.

165. The kit of claim 164, wherein said detectable label is a fluorescent tag.

20 166. The kit of claim 164, wherein said detectable label is a chemiluminescent tag.

167. The kit of claim 164, wherein said detectable label is an enzyme.

25 168. The kit of claim 167, wherein said enzyme is alkaline phosphatase or horseradish peroxidase.

169. The kit of claim 167, further comprising a substrate for said enzyme.

30 170. The kit of claim 161, further comprising:

66670-11872260

- (e) a buffer or diluent; and
- (f) a suitable container means therefor.

5 171. A method for detecting pregnancy in a non-bovine Eutherian animal comprising:

- (a) obtaining a sample from said animal; and
- (b) detecting at least one of pregnancy associated antigen (PAG) in said sample, wherein said PAG is present in early pregnancy,

10 whereby the presence of the PAG indicates that said animal is pregnant.

172. The method of claim 171, wherein said animal is a member of the suborder *Ruminantia*.

15 173. The method of claim 172, wherein said animal is a member of the family *Bovidae*.

174. The method of claim 173, wherein said animal is a goat or sheep.

20 175. The method of claim 174, wherein said animal is a sheep.

176. The method of claim 172, wherein said animal is a member of the order *Perissodactyla*.

25 177. The method of claim 176, wherein said animal is a horse or rhinoceros.

178. The method of claim 177, wherein said animal is a horse.

179. The method of claim 171, wherein said animal is a member of the order *Carnivora.*

180. The method of claim 179, wherein said animal is a dog or cat.

5

181. The method of claim 179, wherein said animal is a human.

Add B3

7